Verified English-Language Translation of U.S. Provisional Application Serial No. 60/349,994

UNITED STATES PATENT AND TRADEMARK OFFICE

I, Susan ANTHONY BA, ACIS,

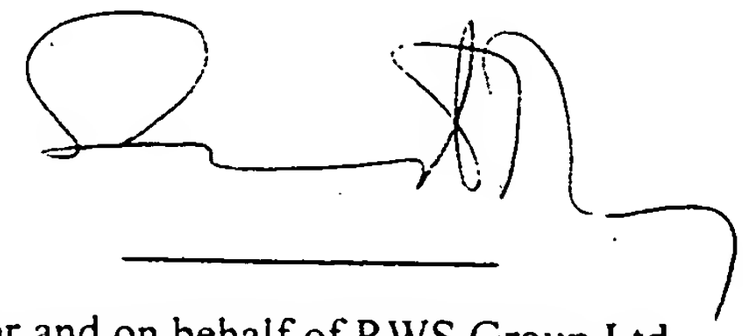
Director of RWS Group Ltd, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That the translator responsible for the attached translation is well acquainted with the German and English languages.

3. That the attached is, to the best of RWS Group Ltd knowledge and belief, a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in the United States of America on 23 January 2002 under the number 60/349,994 and the official certificate attached hereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.



For and on behalf of RWS Group Ltd

The 22nd day of July 2004

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

December 11, 2002

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *60/349,994*
FILING DATE: *January 23, 2002*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

[signature]

L. EDELEN
Certifying Officer

DISINFECTANT/ALCOHOL AMINE II

Description

The invention relates to the use of synergistic disinfectant compositions based on amines and/or quaternary ammonium salts as virucidal agents, in particular towards polioviruses.

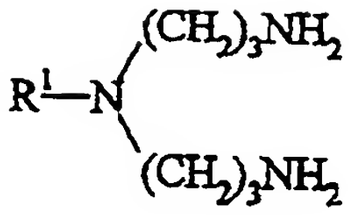
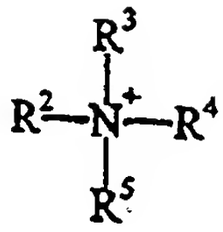
Numerous disinfectant and preservative compositions based on amines and/or quaternary ammonium salts are known. However, in general, in particular at relatively high dilution, these exhibit an unsatisfactory activity towards fungi, for example *Aspergillus niger* and viruses (in particular towards highly resistant viruses, for example polioviruses).

It was therefore an object of the present invention to provide disinfectant compositions based on amines and/or quaternary ammonium salts which exhibit good activity towards fungi and viruses even at high dilution.

This object is achieved according to the invention by the use according to Claim 1.

The earlier application PCT/EP 01/10754 describes disinfectant compositions based on amines and/or quaternary ammonium salts which have good effectiveness towards fungi and viruses even at high dilution.
It has now surprisingly been found that such disinfectant compositions also have pronounced virucidal properties and, in particular, good effectiveness towards highly resistant viruses such as polioviruses.

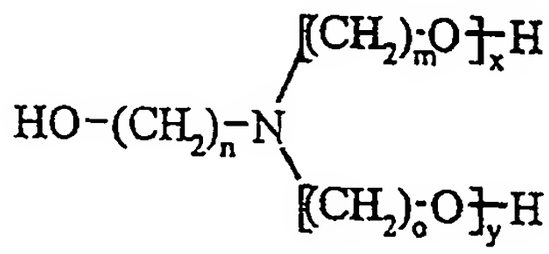
The compositions comprise amines and/or quaternary ammonium salts of the general formula

(Ia) or (Ib),

where R^1 is C_{6-18}-alkyl
R^2 is benzyl or C_{6-18}-alkyl
R^3 is C_{1-18}-alkyl or $-[(CH_2)_2-O]_nR^6$ where n = 1-20
R^4 and R^5 independently of one another are C_{1-4}-alkyl
R^6 is hydrogen or unsubstituted or substituted phenyl
and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid;

and at least one alkanolamine of the general formula



(II),

where n and, if present, m and o independently of one another have the value 2 or 3
and x and y independently of one another have the value 0 or 1, or a corresponding salt; in the mass ratio I:II of 20:1 to 1:20.

Alkyl, here and hereinafter, is taken to mean in each case unbranched or branched alkyl groups of the specified number of carbons, but preferably unbranched alkyl groups, and particularly preferably those having an even number of carbon atoms. In particular, this is also taken to mean the homologue mixtures derived from natural raw materials, for example "coconutalkyl".

Substituted phenyl is taken to mean, in particular, phenyl groups substituted with one or more C_{1-8}-alkyl groups and/or chlorine atoms.

Suitable anions A^- are in principle all inorganic or organic anions, in particular halide, for example

chloride or bromide, or anions of low carboxylic acids, for example acetate, propionate or lactate.

The amine or quaternary ammonium salt (Ia/Ib) is preferably *N,N*-bis(3-aminopropyl)dodecylamine, *N,N*-bis-(3-aminopropyl)octylamine, a didecyldimethylammonium salt, dioctyldimethylammonium salt, octyldecyldimethyl-ammonium salt, dicoconutalkyldimethylammonium salt, coconutalkyldimethylpoly(oxyethyl)ammonium salt, dicoconutalkylmethylpoly(oxyethyl)ammonium salt, decyl-dimethylpoly(oxyethyl)ammonium salt, didecylmethyl-poly(oxyethyl)ammonium salt, octyldimethylpoly(oxy-ethyl)ammonium salt, dioctylmethylpoly(oxyethyl)-ammonium salt, coconutalkyldimethylbenzylammonium salt, benzyldodecyldimethylammonium salt or benzyldimethyl-poly(oxyethyl)ammonium salt or a mixture of two or more of these compounds. Particularly good results were achieved with didecyldimethylammonium salts.

Suitable alkanolamines (II) are in principle all ethanolamines and propanolamines, in particular mono-ethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol. Obviously, using mixtures of the said compounds is also within the scope of the invention. Particularly good results have been obtained using the compounds having a primary amino group, that is to say using monoethanolamine and 3-amino-1-propanol.

The mass ratio of amine (Ia) or quaternary ammonium salt (Ib) to alkanolamine (II) is preferably in the range from 1:5 to 5:1.

The disinfectant compositions used according to the invention preferably comprise water as solvent, if appropriate in combination with an organic solvent.

Preferably, the disinfectant compositions used according to the invention further comprise one or more

aids selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

A preferred use of the disinfectant compositions is surface disinfection and instrument disinfection.

Further preferred fields of use are laundry disinfection and hand disinfection.

A further preferred use of the disinfectant compositions is the use in chemical toilets, for example on board aircraft and vehicles.

The examples below illustrate the implementation of the invention, and should not be taken to be a restriction to the embodiments described. All quantities given, where not otherwise specified, are in % by mass. The test microorganism used in each case was *Aspergillus niger* ATCC 16404. The effectiveness was determined, unless otherwise specified, using the method specified in CEN 1275.

Example 1

A disinfecting cleaner formulation (concentrate) was prepared from:

5.0% didecyldimethylammonium chloride (50% strength solution)
2.0% *N,N*-bis(3-aminopropyl)dodecylamine
5.0% monoethanolamine
5.0% Genapol® T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)
0.5% sodium metasilicate
0.5% sodium carbonate
2.0% methylglycinediacetic acid trisodium salt (Trilon® M; 40% strength solution)
water to 100%

The effectiveness was determined using a dilution (1 part of concentrate, 99 parts of water) at 20°C and with a contact time of 15 min. The logarithm to base ten of the reduction in microorganism count was 4.1.

Comparative Example 1

The procedure of Example 1 was followed, but with the difference that the monoethanolamine was replaced by the same amount of water. Under the same test conditions, the formulation was virtually inactive.

Example 2

A disinfectant formulation (concentrate) was prepared from:

4.9% *N,N*-bis(3-aminopropyl)dodecylamine
4.0% monoethanolamine
2.0% Genapol® T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)
5.0% Hostapur® SAS 30 (C_{13-17} secondary *n*-alkanesulphonic acid, sodium salt)
2.0% ethylenediaminetetraacetic acid tetrasodium salt (40% strength solution)
0.7% ethylenediaminetetraacetic acid
water to 100%

The effectiveness was determined using a dilution (1 part of concentrate, 199 parts of water) at 20°C and with a contact time of 15 min. The logarithm to base ten of the reduction in microorganism count was 4.3.

Example 3

A disinfectant formulation (concentrate) was prepared from:

4.2% *N,N*-bis(3-aminopropyl)dodecylamine
2.0% didecylmethylpoly(oxyethyl)ammonium propionate (BARDAP 26)
4.0% monoethanolamine
2.0% Genapol® T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)
5.0% Hostapur® SAS 30 (C_{13-17} secondary *n*-alkanesulphonic acid, sodium salt)
2.0% ethylenediaminetetraacetic acid tetrasodium salt (40% strength solution)
0.7% ethylenediaminetetraacetic acid
4.0% butyl diglycol
water to 100%

The effectiveness was determined using a dilution (1 part of concentrate, 199 parts of water) at 20°C and with a contact time of 15 min. The logarithm to base ten of the reduction in microorganism count was > 4.4. In addition, the effectiveness was also determined using the method specified in CEN 1650 with a contact time of 15 min, a concentration of 1.0%, a water hardness of 30°fH and an organic load of 0.3% albumin. The logarithm of the reduction in microorganism count was > 4.4.

Examples 4-19

Aqueous solutions were prepared from 0.5% alkanolamine (II) and 0.25% of amine or quaternary ammonium salt (Ia/Ib) and tested using the method specified in CEN 1275. The results are summarized in Table 1.

Table I

Example No.	Amine/ammonium salt	Alkanolamine	log microbial reduction
4	dimethyldioctyl-ammonium chloride	monoethanolamine	4.3
5	ditto	diethanolamine	4.0
6	ditto	triethanolamine	3.6
7	ditto	3-amino-1-propanol	4.2
8	didecyldimethyl-ammonium chloride	monoethanolamine	4.0
9	ditto	diethanolamine	3.8
10	ditto	triethanolamine	3.1
11	ditto	3-amino-1-propanol	4.0
12	di-C_{8-10}-alkyldimethyl-ammonium chloride (60%)/C_{12-16}-alkyl-benzyldimethylammonium chloride (40%); Bardac® 205-M	monoethanolamine	3.9
13	ditto	diethanolamine	3.2
14	ditto	triethanolamine	2.8
15	ditto	3-amino-1-propanol	3.8
16	*N,N*-bis(3-amino-propyl)dodecylamine	monoethanolamine	2.9
17	ditto	diethanolamine	2.7
18	ditto	triethanolamine	2.4
19	ditto	3-amino-1-propanol	2.8

For comparison, all compounds listed in Table 1 were tested as individual substances in 0.5% strength solution. None of these compounds exhibited pronounced fungicidal activity (log microbial reduction < 2).

Example 20

A disinfectant formulation (concentrate) was produced from:

9.9% didecyldimethylammonium chloride (70% strength solution)
8.0% monoethanolamine
5.0% Genapol®T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)
5.0% potassium carbonate (anhydrous)
6.0% ethylenediaminetetraacetic acid tetrasodium salt (Trilon®B; 40% strength solution)
water to 100%

Example 21

The concentrate described in Example 20 was tested in 6% strength dilution in the suspension test using an exposure time of 30, 60 and 120 minutes for effectiveness against poliovirus type 1 (Mahoney strain).

Test method:

The test was performed in accordance with the "Richtlinie des Bundesgesundheitsamtes und der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren" [Guideline of the German Federal Health Agency and the German Association for Controlling Viral Diseases for testing chemical disinfectants for effectiveness against viruses] (*Bundesgesundheitsbl.* **1982**, *25*, 397). The growth medium for the Vero cell cultures was "Dulbecco's Modified Eagle's Medium", to which 10% foetal calf serum and 10 U/ml of penicillin and also 10 µg/ml of streptomycin had been added. After the tissue culture was inoculated with poliovirus the tissue culture medium only contained 3% foetal calf

serum. After virtually complete detachment of polio-infected cells, the virus suspension was purified by centrifuging of cells and cell constituents (3000 × g, 15 min). Since the cell culture medium contained 3% foetal calf serum, in the disinfectant test also, a small protein load was also present in the test batches using twice-distilled water.

For the disinfectant test, 1 part of virus suspension was mixed with 8 parts of a 7% strength dilution of the disinfection concentrate (corresponding to a final concentration of 6%) and in each case 1 part of twice-distilled water or 2% strength serum albumin or foetal calf serum and was incubated for 30, 60 and 120 min at 20°C. The activity of the disinfectant was then stopped by 100-fold dilution with cold medium containing no foetal calf serum. In each case 2 wells of multiwell plates containing 6 recesses (Becton Dickinson Labware, Lincoln Park, NJ, Type Falcon™353046) which contained a dense lawn of Vero cells, were inoculated with 1 ml in each case of this dilution (corresponding to a dilution of the virus suspension to 10^{-3}) and further serial 10-fold dilutions. After 1 h of adsorption time at room temperature, the supernatant liquid was drawn off. The cell lawns of the wells were then coated with 2 ml of 2% strength agarose (Serva high EEO, Cat. No. 11397) liquefied by boiling, which had been mixed with twice-concentrated medium containing 5% strength foetal calf serum in a ratio of 1:1, and had been cooled to 40°C in a waterbath. After solidification of the agarose at room temperature, the plates were incubated for 2 days at 37°C in a CO_2 incubation cabinet.

The infectivity of the virus suspension was tested in the plaque test. In this test each area of destroyed cells corresponds to one infectious unit of poliovirus. The number of plaques thus indicates the number of infectious virus particles present in a defined dilution of the test batch. The plaques are visualized by staining 1.0 ml in each case of a solution of 0.1% Brilliant Blue R (Sigma, Cat. No. B0149) for 30 min in

an aqueous solution containing 20% methanol and 5% acetic acid. The unstained plaques are then clearly differentiated from blue-coloured cell lawns. A mean plaque count is calculated from two batches in each case of a dilution.

"Virus controls", in which the starting concentration of the virus was determined, were batches in which the disinfectant had been replaced by the same volume of twice-distilled water. The virus concentration thus determined served as reference for calculating the virus-inactivating action of the disinfectant tested.

"Toxicity controls" for detecting any damage of the tissue culture cells by the disinfectant were batches in which the virus suspension had been replaced by the same volume of twice-distilled water. These batches were diluted in a ratio of 1:100 and 1:1000 (equivalent to a dilution of the virus suspension of 10^{-3} and 10^{-4} in the disinfectant test batch) with medium without foetal calf serum. Then they were added to the tissue culture, as with the batches for testing the disinfectant action, for 1 h and then drawn off. After incubation for 2 days at 37°C, staining was used to test whether the cell lawn had been damaged by the disinfectant.

As an indication of the resistance of the test virus and for comparability with other studies, a "formaldehyde control" was carried out. For this, 1 part of the virus suspension was mixed with 4 parts of phosphate-buffered saline (0.1 M; pH 7; "Dulbecco's PBS") and the entire volume was added to a formalin solution containing 1.4 g of formaldehyde in 100 ml of solution (final concentration: 0.7 g of HCHO/100 ml). After 5, 15 and 60 min of exposure time, the action of formaldehyde was stopped, as with the disinfectant test, by diluting to 1:100 and the remaining infectivity of the poliovirus was determined in the plaque test in further serial ten-fold dilutions.

Results:

Control experiments:

The "virus control", in the batch with twice-distilled water, gave a virus concentration of $1.6 \cdot 10^3$ infectious units/ml, in the batch containing serum albumin, $1.2 \cdot 10^8$ infectious units/ml, and in the batch containing foetal calf serum $1.0 \cdot 10^8$ infectious units/ml. The "toxicity control", after dilution of the test batch to 1:100 (equivalent to a dilution of the virus suspension of 10^{-3}) showed slight damage of the cell lawn. At a dilution of 1:1000, toxicity was no longer observable. Thus under the test conditions, a decrease in virus concentration under the action of disinfectant can be followed to a virus concentration of $5 \cdot 10^3$ infectious units/ml in the virus suspension (in both wells of the dilution 10^{-4}, plaque is then no longer visible) and at a starting concentration of at least 10^8 infectious virus particles/ml, a decrease in virus concentration over at least 4.5 powers of ten is observable. Since the test guideline for detecting the effectiveness of a disinfectant only requires a decrease in virus concentration by at least 4 powers of ten, compliance with this condition can be detected using the experimental batch chosen.

In the batch containing 0.7% strength formaldehyde, after an exposure time of 5 min, a virus concentration of $1.05 \cdot 10^6$/ml was measured, after 15 min $1 \cdot 10^3$/ml, and after 60 min $\leq 5 \cdot 10^2$/ml. These are expected values which confirm the results of earlier experiments: 0.7% strength formalin is usually able to reduce the concentration of poliovirus by more than 4 powers of ten within 30 min.

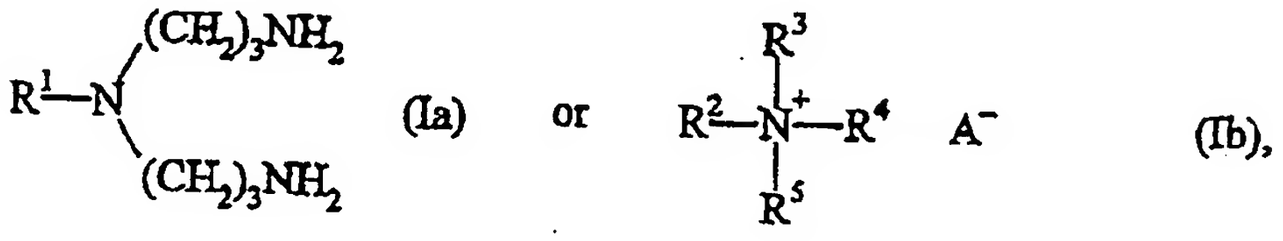
Effectiveness of the disinfectants against poliovirus:

After 30, 60 and 120 min exposure times of 6% strength dilution of the disinfectant composition from Example 20, in the batch containing foetal calf serum at the virus dilution 10^{-4}, in each of the two test wells plaque was no longer observed. Thus after the disinfectant treatment, a virus concentration of $\leq 5 \cdot 10^3$

infectious units/ml was present. This result was found not only with low protein load (batch with twice-distilled water), but also with medium (batch containing 2% strength serum albumin) and high protein load (batch containing foetal calf serum). Thus, compared with the control determination without disinfectant, there was a decrease in virus concentration by at least 4.5 $\log_{10}$ or powers of ten. Thus the condition for effectiveness for registration as instrument disinfectant in the Federal Republic of Germany is fulfilled.

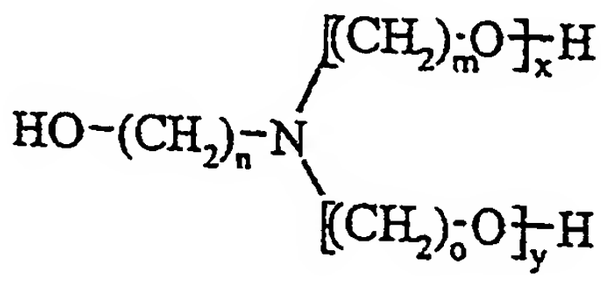
Claims

1. Use of a disinfectant composition comprising
   a) an amine and/or quaternary ammonium salt of the general formula



(Ia) or (Ib),

where R^1 is C_{6-18}-alkyl
R^2 is benzyl or C_{6-18}-alkyl
R^3 is C_{1-18}-alkyl or $-[(CH_2)_2-O]_nR^6$ where n = 1-20
R^4 and R^5 independently of one another are C_{1-4}-alkyl
R^6 is hydrogen or unsubstituted or substituted phenyl
and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid; and
   b) at least one alkanolamine of the general formula



(II),

where n and, if present, m and o independently of one another have the value 2 or 3
and x and y independently of one another have the value 0 or 1, or a corresponding salt;
in the mass ratio I:II of 20:1 to 1:20 as virucidal agent.

2. Use according to Claim 1, characterized in that the amine or quaternary ammonium salt is selected from the group consisting of *N,N*-bis-

(3-aminopropyl)dodecylamine, *N*,*N*-bis(3-aminopropyl)octylamine, didecyldimethylammonium salts, dioctyldimethylammonium salts, octyldecyldimethylammonium salts, coconutalkyldimethylbenzylammonium salts and benzyldimethyloxoethylammonium salts and mixtures of these compounds.

3. Use according to Claim 1 or 2, characterized in that the alkanolamine (II) is selected from the group consisting of monoethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol.

4. Use according to one of Claims 1 to 3, characterized in that the mass ratio I:II is between 1:5 and 5:1.

5. Use according to one of Claims 1 to 4, characterized in that the disinfectant composition comprises water as solvent.

6. Use according to one of Claims 1 to 5, characterized in that the disinfectant composition additionally comprises one or more aids selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

7. Use according to one of Claims 1 to 6 in surface disinfection and instrument disinfection.

8. Use according to one of Claims 1 to 6 in laundry disinfection.

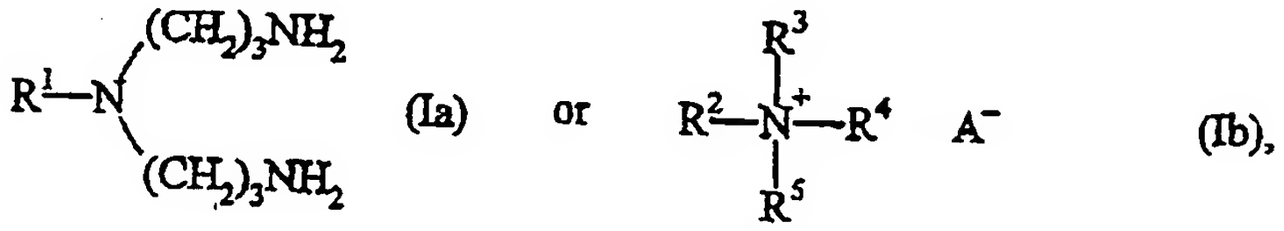
9. Use according to one of Claims 1 to 6 in hand disinfection.

10. Use according to one of Claims 1 to 6 in chemical toilets.

Abstract

A description is given of the use of disinfectant compositions comprising

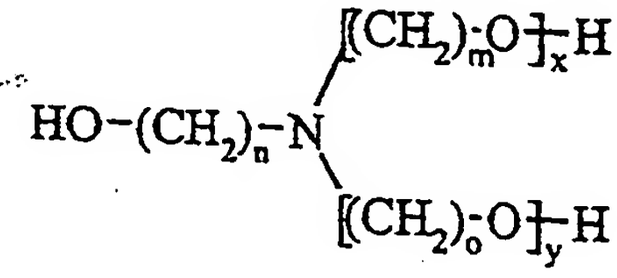
a) at least one amine and/or quaternary ammonium salt of the general formula



(Ia) or (Ib),

where R^1 is C_{6-18}-alkyl
R^2 is benzyl or C_{6-18}-alkyl
R^3 is C_{1-18}-alkyl or $-[(CH_2)_2-O]_nR^6$ where n = 1-20
R^4 and R^5 independently of one another are C_{1-4}-alkyl
R^6 is hydrogen or unsubstituted or substituted phenyl
and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid; and

b) at least one alkanolamine of the general formula



(II),

where n and, if present, m and o independently of one another have the value 2 or 3
and x and y independently of one another have the value 0 or 1, or a corresponding salt;

in the mass ratio I:II of 20:1 to 1:20 as virucidal agent, in particular towards polioviruses. The compositions are also distinguished by good bactericidal, and in particular also fungicidal,

effectiveness, even at low usage concentrations.

Basle, 18 January 2002
Dr N. Riegler